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**Title:**

Regulation of Adipose Cell Growth and Turnover, and Its Dysfunction in Insulin Resistance\*

**Abstract:**

The biological mechanism by which obesity predisposes to insulin resistance is unclear. While adipose cell size is known to increase in proportion to BMI, it has not been clearly shown that cell size, independent of BMI, is associated with insulin resistance. Here we report the results of several studies aimed at reexamining this relationship.

Initially, we compared adipose cell size distribution in 28 equally obese, otherwise healthy individuals who represented extreme ends of the spectrum of insulin sensitivity, as defined by the modified insulin suppression test. All individuals exhibited a non-unimodal cell size distribution featuring an approximately normal peak of large cells and a tail of small cells. Contrary to expectations, the mean diameter of the larger cells was not significantly different between the insulin-sensitive and insulin-resistant individuals, but rather insulin resistance was associated with a higher ratio of small to large cells. Similar cell size distributions were observed for isolated adipose cells. Further, real-time PCR results showed two- to threefold lower expression of genes encoding markers of adipose cell differentiation in insulin-resistant compared with insulin-sensitive individuals. These results demonstrate that after controlling for obesity, insulin resistance is associated with an expanded population of small adipose cells and decreased expression of differentiation markers, suggesting that impairment in adipose cell differentiation may contribute to obesity-associated insulin resistance.

We then sought to determine whether increased adipose cell size is associated with localized inflammation in weight-stable, moderately obese humans. We recruited 49 healthy, moderately obese individuals for quantification of insulin resistance (modified insulin suppression test) and subcutaneous abdominal adipose tissue biopsy. Adipose cells were again non-unimodally distributed, with 47% in a 'large' cell population and the remainder in a 'small' cell population. The median diameter of the large adipose cells was not associated with expression of inflammatory genes. Rather, the fraction of small adipose cells was consistently associated with inflammatory gene expression, independently of sex, insulin resistance and BMI. This association was more pronounced in insulin-resistant than insulin-sensitive individuals. Insulin resistance also independently predicted expression of inflammatory genes. This study demonstrates that among moderately obese, weight-stable individuals an increased proportion of small adipose cells is associated with inflammation in subcutaneous adipose tissue, whereas size of mature adipose cells is not. The observed association between small adipose cells and inflammation may reflect impaired adipogenesis and/or terminal differentiation.

Another study was initiated to compare the characteristics of adipose cells in subcutaneous and omental visceral adipose tissue (SAT and VAT, respectively); in this instance, individuals were chosen to be insulin-resistant, but varied in degree of adiposity. We compared adipose cell size distribution and genetic markers, in SAT and VAT of individuals undergoing bariatric surgery. While the proportion of small cells and expression of adipocyte differentiation genes did not differ between depots, inflammatory genes were upregulated in VAT. The diameter of SAT large cells correlated highly with increasing proportion of small cells in both SAT and VAT. No associations were observed between VAT large cells and cell size variables in either depot. The

effect of body mass index (BMI) on any variables in both depots was negligible. Thus, the major differential property of VAT in IR women is increased inflammatory activity, independent of BMI. The association of SAT adipocyte hypertrophy with hyperplasia in both depots suggests a primary role SAT may have in regulating regional fat storage.

Finally, we carried out a study in human subjects to determine whether pioglitazone stimulates adipogenesis *in vivo* and whether this process relates to improved insulin sensitivity. To test this hypothesis, 12 overweight/obese nondiabetic, insulin-resistant individuals underwent biopsy of abdominal subcutaneous adipose tissue at baseline and after 12 weeks of pioglitazone treatment. Insulin resistance (steady-state plasma insulin and glucose (SSPG)) decreased following pioglitazone treatment. There was an increase in the ratio of small-to-large adipose cells, as well as a 25% increase in the absolute number of small adipose cells. The distribution of large cell diameters widened, but the diameter did not increase in the case of the small cells. The increase in the proportion of small cells was associated with the degree to which insulin resistance improved. Visceral abdominal fat decreased, and subcutaneous abdominal and femoral fat increased significantly. Changes in fat volume were not associated with SSPG change. These findings demonstrate a clear effect of pioglitazone on human subcutaneous adipose cells, suggestive of adipogenesis in abdominal subcutaneous adipose tissue, as well as redistribution of fat from visceral to subcutaneous depots, highlighting a potential mechanism of action for thiazolidinediones. These findings support the hypothesis that defects in subcutaneous fat storage may underlie obesity-associated insulin resistance.

\*In collaboration with T. M. McLaughlin<sup>1</sup>, P. Tsao<sup>1</sup>, G.M. Reaven<sup>1</sup>, A. Sherman